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(57) Abstract

New human nuclear steroid hormone receptor antagonist co-modulator molecules that bind receptor-antagonist complex are provided. The molecules of the invention are useful for screening steroid receptor ligand antagonists and for modulating the *in vivo* activity of steroid antagonists.

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STEROID RECEPTOR ANTAGONIST CO-MODULATOR AND METHODS FOR USING SAME

FIELD OF THE INVENTION

This invention relates to the field of steroid hormones, specifically, steroid receptor binding proteins and assay methods for identifying steroid antagonists.

BACKGROUND OF THE INVENTION

Members of the nuclear hormone receptor superfamily includes the receptors for steroid hormones, thyroid hormones, lipophilic vitamins such as vitamins A and D, and the orphan receptors, which have a structure consistent with other superfamily members but have no identified ligands (Evans (1988) Science 240:889-895). The receptors regulate gene expression by interacting with specific DNA sequences (hormone response elements, or "HREs") in the promoters of target genes (Glass (1994) Endocr. Rev. 15:391-407).

Nuclear receptors are grouped into two subfamilies: the thyroid/retinoic acid/vitamin D receptor (TRV) family and the steroid receptor (R_s) family. Steroid hormone receptors bind to their respective HREs in a ligand-dependent manner whereas some receptors such as the thyroid hormone receptor (T_3R) and retinoic acid receptor (RAR/RXR) bind to their response elements in a ligand-independent manner. In the absence of ligand, TRV receptors bind DNA at HREs to actively repress transcription of their target genes. In contrast to the TRV mechanism, unactivated, ligand-free steroid receptors do not bind DNA. In the presence of a ligand, however, dimeric steroid receptors bind to HREs present in the promoters of genes to regulate gene transcription.

Recently, three studies have described mouse proteins which act as a co-repressor of ligand-independent T_3R/RAR repression of transcription (Hörlein et al. (1995) Nature 377:397-403; Chen & Evans (1995) Nature 377:454-457; Burris et al. (1995) Proc. Natl. Acad. Sci. 92:9525-9529). These co-repressors specifically bind with DNA-bound T_3R or RAR in the absence of ligand, but not in its presence. These co-repressors fail to interact with unliganded members of the steroid receptor subfamily.

Steroid receptors play a role in normal health and in a spectrum of disease states, including cancer, inflammation, endocrine disorders, and oral contraception. The natural steroid hormones produced by endocrine glands bind to steroid hormone receptors in target organs. The natural steroid hormones include estrogens, progestins, androgens, glucocorticoids and mineralocorticoids. These hormones are defined as agonists, and hormone-receptor complexes modulate specific gene transcription by either increasing or decreasing transcription rate. Steroid agonists have pleiotropic physiological actions in a number of tissues, for example, estradiol and progesterone regulate gene transcription in the kidney, ovary, cervix, uterus, bone, skin, breast, heart, pituitary and brain.

Hormones of the steroid receptor subfamily are used to treat many disorders and are used in healthy people for oral contraception and hormone replacement therapy, among others. It is often medically desirable to block the actions of steroid hormone agonists. For this reason, researchers have synthesized steroid receptor antagonists that are used in breast, endometrial and prostate cancer treatment as agents to prevent cancer development or block abnormal growths, and as contraceptive agents. These antagonists ligands also

bind to the steroid receptors, but in general, they have effects that are opposite to the ones produced by the agonists.

The actions of steroid receptor antagonist are complex. They often have dual agonist/antagonist effects. For example, an antagonist may have 5-10% of the biological activity of an agonist; thus, the antagonist may block the activity of the agonist, resulting in substantially decreased agonist activity. Antagonists may also have the desired antagonist effect in one tissue (for example, the breast), but may have an agonist effect in another tissue (for example, the uterus). The agonist effect of an antagonist may or may not be an unwanted side-effect. Similarly, in cancer treatment, an antagonist ligand may initially have the desired inhibitory effect on the tumor, but with time, the ligand switches to an agonist-like effect and the cancer then resumes growing. This is clearly a dangerous side-effect, often described as "resistance" to treatment.

Steroid receptor co-repressor molecules have not been previously described. Recently, a series of novel nuclear co-activator proteins have been identified that interact with the ligand-activated hormone binding domain (HBD) of steroid receptors and enhance their transcriptional activity by as yet unknown mechanisms (Halachmi et al. (1994) Science 264:1455-1458; Cavaillès et al. (1994) Proc. Natl. Acad. Sci. 91:10009-10013; Baniahmad (1995) Mol. Endocrinol. 9:34-43).

SUMMARY OF THE INVENTION

A new group of human nuclear steroid hormone receptor molecules have been identified that interact with antagonist-occupied nuclear receptors, and are termed "human nuclear receptor antagonist co-modulators"

("hNRA-coM"). These molecules, described herein, are encoded by the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO;12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18 (SEQ ID NOs:1-18). The hNRA-coM molecules encoded by the nucleotide sequences of SEQ ID NOs:1-18 specifically bind a nuclear steroid hormone receptor-antagonist complex and mediate the biological activity of the antagonist-bound receptor. The hNRA-coMs of the invention do not bind a nuclear steroid hormone receptor-agonist complex.

Accordingly, the invention features a substantially pure human nuclear steroid hormone receptor co-modulator molecule encoded by any one of the nucleotide sequences of SEQ ID NOs:1-18 and characterized as binding a nuclear steroid hormone receptor-antagonist complex.

The invention includes the specific proteins disclosed, as well as closely related proteins and peptides that are identified and isolated by the use of probes or antibodies prepared from the nucleotide and amino acid sequences herein disclosed. This can be done using standard techniques, e.g., by screening a genomic cDNA or combinatorial chemical library with a probe having all or part of the nucleotide sequence of the disclosed hNRA-coM proteins. The invention further includes synthetic polypeptides having all or part of the sequences of the hNRA-coM molecules disclosed herein. hNRA-coM molecules include functional fragments of the proteins and peptides shown, as long as the activity of hNRA-coM molecule, e.g., its ability to bind a steroid receptor-antagonist complex, remains. Smaller peptides

containing the biological activity of hNRA-coM are included in the invention.

In one aspect, the invention features isolated and purified nucleotide sequences which encode the hNRA-coM molecules of the invention. In one embodiment, the nucleotide sequence is a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-18. In a preferred embodiment, the nucleotide sequence has the nucleotide sequence of SEQ ID NO:17 or SEQ ID NO:18. Most preferably, the nucleotide sequence is the nucleotide sequence comprising both SEQ ID NO:17 and SEQ ID NO:18. As shown in Fig. 2, the nucleotide sequences of SEQ ID NOs:17-18 encode different portions of a single hNRA-coM peptide molecule which binds antagonist-occupied nuclear steroid hormone receptor.

The hNRA-coM molecules of the invention bind a steroid hormone receptor when the ligand binding site is occupied by an antagonist, but not when the ligand binding site is occupied by an agonist. Thus, the hNRA-coM molecules of the invention are useful to screen candidate nuclear steroid hormone receptor ligands which are potential steroid antagonists.

Accordingly, in one aspect, the invention features a method for identifying potential steroid receptor antagonists by contacting a steroid receptor with a test ligand, such that a receptor-ligand complex is formed, contacting the receptor-ligand complex with a hNRA-coM molecule, and detecting hNRA-coM binding to the receptor-ligand complex. Binding of hNRA-coM molecule to the complex indicates that the bound ligand is a candidate steroid antagonist. Absence of binding indicates that the bound ligand is not a steroid antagonist, and may be a steroid agonist.

The hNRA-coM molecules of the invention modulate the biological activity of a steroid receptor-antagonist

complex by blocking the agonist biological activity of the steroid antagonist and promoting the antagonist activity. Thus, the hNRA-coM molecules of the invention are therapeutically useful for treating medical conditions related to steroid antagonist treatment, and are useful for eliminating unwanted agonist side-effects resulting from steroid antagonist treatments.

Accordingly, in one aspect the invention features a method of providing hNRA-coM molecules to a subject in need of steroid antagonist treatment in order to block the undesirable agonist effect of a steroid antagonist. In one embodiment of the treatment method of the invention, a hNRA-coM molecule can be provided to a specific cell, tissue, or organ target where it is desirable to block the agonist action of a steroid antagonist. Methods of targeting compounds to a specific site in the body are known in the art. In another embodiment, portions of DNA encoding an hNRA-coM molecule may be introduced into cells that underexpress an hNRA-coM protein. In a further aspect, the expression of an endogenous hNRA-coM molecule may be increased by administration of an agent which enhances hNRA-coM gene transcription.

In another aspect, the hNRA-coM molecule of the invention is therapeutically useful in increasing the agonist action of an steroid antagonist when the agonist action of the steroid antagonist is blocked by the presence of endogenous hNRA-coM molecule. In such a case, the expression of endogenous hNRA-coM molecule may be removed by administration of a therapeutic reagent which decreases the level of hNRA-coM molecule transcription or translation. Nucleic acid sequences that interfere with hNRA-coM expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid, ribozymes, or

triplex agents to block transcription or translation of hNRA-coM mRNA, either by masking the mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme. In a specific embodiment, the therapeutic agent is an antisense polynucleotide able to bind hNRA-coM protein mRNA. In another embodiment, the therapeutic agent is an antibody that specifically binds the endogenous hNRA-coM molecule thus inhibiting hNRA-coM binding to a nuclear receptor-antagonist complex. The therapeutic agent can be administered in site-specifically where needed, e.g., targeted to a specific tissue. In a further aspect, the expression of an endogenous hNRA-coM molecule may be inhibited by administration of an agent which inhibits hNRA-coM gene transcription.

In a related aspect, the hNRA-coM molecules of the invention are therapeutically useful in modulating the biological activity of a dual action antagonist (e.g., an antagonist having partial agonist activity). To enhance the antagonist effects of the ligand, the cellular availability of the hNRA-coM molecule to the ligand/receptor complex is increased; to enhance the agonist effects of the ligand, the availability of the hNRA-coM to the ligand/receptor complex is decreased.

One advantage of the invention is that the hNRA-coM molecules of the invention can be used to identify candidate steroid antagonists. Another advantage is that the invention provides a rapid and convenient assay for identifying steroid antagonists.

In addition to these advantages of the use of hNRA-coM molecules, other advantages and features of the present invention will become apparent to those skilled in the art upon reading this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1R shows partial nucleotide sequences for a human nuclear steroid receptor antagonist co-modulator molecules (hNRA-coM).

Fig. 2 is a schematic diagram of the hNRA-coM DNA sequence TJ53 (SEQ ID NOs:17-18) aligned to the mouse TRV receptor co-repressor sequence.

Fig. 3 is a schematic representation of the interaction between the "bait" construct (LexA:PR HBD fusion protein) and protein X:Gal activation domain fusion protein in the yeast two hybrid system. The interaction between the bait construct and protein X results in expression of histidine (growth selection) and β-galactosidase (blue color).

DETAILED DESCRIPTION

Before the methods and compositions of the present invention are described and disclosed it is to be understood that this invention is not limited to the particular methods and compositions described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a human nuclear steroid receptor antagonist co-modulator (hNRA-coM molecule)" includes a plurality of hNRA-coM proteins and peptides able to bind a steroid antagonist-receptor complex.

Unless defined otherwise all technical and scientific terms used herein have the same meaning as

commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials or methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the particular information for which the publication was cited in connection with.

Definitions

By the term **"hNRA-coM molecule"** is meant a molecule encoded by any one of the nucleotide sequences of SEQ ID NOs:1-18 which is characterized by its ability to bind an antagonist-occupied nuclear steroid receptor. In one embodiment, an hNRA-coM molecule shall mean a naturally occurring, recombinant, or synthetic full length protein or fragment thereof encoded by a nucleotide sequence substantially similar (e.g., 90% or greater homology) to a portion of the nucleotide sequences of SEQ ID NOs:1-18, and capable of binding the antagonist-occupied steroid receptor complex. In a preferred embodiment, an hNRA-coM molecule shall mean a naturally occurring, recombinant, or synthetic amino acid sequence encoded by a nucleotide sequence substantially similar (e.g., 90% or greater homology) to a portion of the naturally occurring hNRA-coM molecule encoded by the nucleotide sequence of SEQ ID NO:17 and/or SEQ ID NO:18, and able to bind the antagonist-occupied steroid receptor complex.

By **"steroid receptor"** or **"nuclear steroid receptor** is" meant a protein that is a ligand-activated transcription factor, and belongs to the steroid receptor subfamily of nuclear receptors. Included in the definition of steroid receptors are proteins which

structurally resemble and have the biological activity of a steroid hormone-activated transcription factor. Steroid receptors contain all or part of a DNA binding domain and a hormone (or ligand) binding domain, and include orphan receptors for unknown ligands whose structure resembles that of steroid receptors.

By "**steroid receptor ligand**" is meant a natural or synthetic compound which binds the nuclear steroid receptor to form a receptor-ligand complex. The term ligand includes agonists, antagonists, and compounds with partial agonist/antagonist action.

By "**steroid receptor agonist**" is meant a compound which binds the nuclear steroid receptor to form a receptor-agonist complex. The receptor-agonist complex binds specific regions of DNA termed hormone response elements (HREs). Natural steroid hormone agonists include estradiol, progesterone, androgens, glucocorticoids, and mineralocorticoids.

By "**steroid receptor antagonist**" is meant a compound that has a biological effect opposite to that of an agonist. An antagonist binds the nuclear steroid receptor and blocks the action of a steroid receptor agonist by competing with the steroid agonist for receptor. An "antagonist" is defined by its ability to block the actions of an agonist. Steroid receptor antagonists include "pure" antagonists, as well as compounds with partial agonist/antagonist action. A pure antagonist effectively competes with an agonist for receptor binding, without itself having agonist actions. A partial antagonist may be less effective at competing with an agonist for receptor binding, or may be equally effective at binding the receptor but have only 5-10% of the agonist action than that of the agonist being competed with. Thus, an antagonist may have an agonist effect less effective than that of the competing agonist.

By the term "**nucleotide sequence**" is meant a sequence of deoxyribonucleotides or ribonucleotides in the form of a separate fragment or as a component of a larger construct. DNA encoding portions or all of the proteins of the invention can be assembled from cDNA fragments or from oligonucleotides that provide a synthetic gene which can be expressed in a recombinant transcriptional unit. Nucleotide sequences of the invention include DNA, RNA, and cDNA sequences, and can be derived from natural sources or synthetic sequences synthesized by methods known to the art.

By the term "**isolated**" nucleotide sequence is meant a nucleotide sequence that is not immediately contiguous (i.e., covalently linked) with either of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the nucleotide sequence is derived. The term therefore includes, for example, a recombinant nucleotide sequence which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

The isolated and purified nucleotide sequences of the invention also include nucleotide sequences that hybridize under stringent conditions to the nucleotide sequences specified herein. The term "**stringent conditions**" means hybridization conditions that guarantee specificity between hybridizing nucleotide sequences, such as those described herein, or more stringent conditions. One skilled in the art can select posthybridization washing conditions, including temperature and salt concentrations, which reduce the

number of nonspecific hybridizations such that only highly complementary sequences are identified (Sambrook et al. (1989) in Molecular Cloning, 2d ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, hereby specifically incorporated by reference).

The isolated and purified nucleotide sequences of the invention also include sequences complementary to the nucleotide encoding hNRA-coM molecules (antisense sequences). Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub (1990) Scientific American 262:40). The invention includes all antisense nucleotide sequences capable of inhibiting production of hNRA-coM proteins. In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and introduced into a target hNRA-coM-producing cell. The use of antisense methods to inhibit the translation of genes is known in the art, and is described, e.g., in Marcus-Sakura (1988) Anal. Biochem. 172:289.

In addition, ribozyme nucleotide sequences for hNRA-coM molecules are included in the invention. Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech (1988) J. Amer. Med. Assn. 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

The hNRA-coM molecules of the invention can also be used to produce antibodies that are immunoreactive or bind epitopes of the hNRA-coM molecules. Accordingly, one aspect of the invention features antibodies to the hNRA-coM molecules of the invention. The antibodies of the invention include polyclonal antibodies which consist of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from antigen-containing fragments of the hNRA-coM molecules by methods known in the art (See, for example, Kohler et al. (1975) Nature 256:495).

The term "**antibody**" as used herein includes intact molecules as well as fragments thereof, such as Fa, $F(ab')_2$, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind hNRA-coM molecules can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from translated cDNA or chemically synthesized, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit). Antibodies produced by recombinant methods known in the art are also included (Russell et al. (1993) Nucleic Acid Res. 21:1081-1085; McCafferty et al. (1990) Nature 348:552-554; Dueñas & Borrebaeck (1994) Bio/Technology 12:999-1002).

By the term "**substantially pure**" is meant a compound, such as an hNRA-coM molecule, which is substantially free of other proteins, lipids, carbohydrates, or other materials with which it is naturally associated. A substantially pure hNRA-coM

molecule is at least 60%, by weight, free of other components, more preferably at least 75%, even more preferably at least 90%, and most preferably at least 99%, by weight, hNRA-coM protein. One skilled in the art can purify hNRA-coM using standard techniques for protein purification, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding a hNRA-coM molecule or by chemically synthesizing the protein. The substantially pure protein will yield a single band on a non-reducing polyacrylamide gel. The purity of the hNRA-coM molecule can be determined by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, HPLC analysis, and/or amino-terminal amino acid sequence analysis.

By the term "**host cell**" is meant a cell in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer in which the exogenous DNA is continuously maintained in the host are known in the art. Suitable host cells for use in the invention include microbial, yeast, insect, and mammalian organisms.

By the term "**recombinant expression vector**" is meant a plasmid, virus, or other vehicle known in the art that has been manipulated by insertion or incorporation of the hNRA-coM DNA sequences. The DNA sequence can be present in the vector operably linked to regulatory elements, for example, the promoter. Such expression vectors contain a promoter sequence which facilitates the efficient transcription in the host of the inserted nucleic acid sequence. The expression vector typically

contains an origin of replication, a promoter, as well as specific nucleotide sequences which allow phenotypic selection of the transformed cells. For the host cell, the expressed nucleotide sequence is also, if desired, translated into the hNRA-coM protein.

The terms "**treatment**", "**treating**", and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptoms thereof and/or may be therapeutic in terms of a partial or complete cure for a disease or medical condition and/or adverse effect attributable to a medical condition. More specifically, "treatment" is intended to mean providing a therapeutically detectable and beneficial effect of a patient suffering from condition resulting from a steroid antagonist.

By the term "**therapeutic reagent**" is meant a compound or molecule that achieves the desired effect on an steroid antagonist-related condition when administered to a subject in need thereof.

By the term "**therapeutically effective amount**" is meant an amount of a reagent sufficient to decrease, prevent or enhance the symptoms associated with a steroid antagonist-related condition.

By the term "**steroid antagonist-related condition**" or "**antagonist-related condition**" is meant a medical condition resulting, at least in part, from antagonist binding to nuclear steroid receptors to form an antagonist-steroid receptor complex. For example, an antagonist-related condition may result from administration of a progesterone antagonist (or antiprogestin) to a healthy female subject for use as a contraceptive through the ability of the antiprogestin to block the actions of progesterone at the ovary and

uterus. Similarly, an antagonist-related condition includes the therapeutic use of an antiprogestin in a female subject with breast cancer to block the growth-promoting effect of progesterone at the tumor. In both cases, the antiprogestin block of progesterone action at a specific site may be undesirable, e.g., at the bone. The hNRA-coM molecules of the invention are useful to modulate the undesirable antagonist effects of antiprogestins at specific sites such as the bone.

An antagonist-related condition may result when an antagonist is intentionally administered therapeutically, for example, when an active antagonist is prevented from having its desired biological effect at a target tissue because of the presence of endogenous hNRA-coM protein.

The term "**modulation of antagonist activity**" may include inhibitory or stimulatory effects, where the effect is opposite to that effect produced by an agonist. The present invention is particularly useful for enhancing steroid antagonist activity by providing a hNRA-coM molecule in cells underexpressing hNRA-coM, or for reducing antagonist activity by blocking hNRA-coM molecule expression in cells overexpressing hNRA-coM, or in which any expression of hNRA-coM is undesirable.

By the term "**enhancing steroid antagonist activity**" is meant producing a stronger or more specific antagonist effect.

By the term "**hNRA-coM dominant negative mutant**" is meant a hNRA-coM variant having one or more mutations in its encoding nucleotide sequence relative to the natural sequence, which binds to the antagonist-occupied receptor complex but does not enhance antagonist activity. Thus, a dominant negative mutant hNRA-coM molecule would compete with the endogenous protein for binding to the antagonist-occupied receptor complex, inhibiting or

decreasing the biological effectiveness of the endogenous protein.

By the term "**hNRA-coM dominant positive mutant**" is meant a hNRA-coM variant having one or more mutations in its encoding nucleotide sequence relative to the natural sequence, which binds to the antagonist-occupied receptor complex and enhances antagonist activity to a greater extent than the natural molecule.

By the term "**reducing**" or "**inhibiting**" steroid antagonist activity is meant producing a weakened antagonist effect or eliminating an antagonist effect.

The Human Nuclear Steroid Receptor Antagonist Co-Modulator (hNRA-coM) Molecules

The invention provides substantially pure human nuclear steroid receptor antagonist co-modulator protein and peptide molecules (hNRA-coMs), characterized as cellular proteins or peptides which bind the nuclear steroid receptor-antagonist complex.

The hNRA-coM molecules of the invention are comprised of the nucleotide sequences of SEQ ID NOs:1-18. As described in the experiments below, LexA/DNA-binding domain-progesterone receptor fusion proteins were used in a generic two-hybrid screen to isolate progesterone receptor interactors expressed in yeast from a HeLa cell library (Example 1). Progesterone is a key hormone involved in the development, growth and maintenance of female reproductive function. Progesterone antagonists block the progesterone receptor signal transduction pathway. They are therefore indicated for pregnancy termination, for contraception, for the treatment of endometriosis and uterine fibroids, and for the therapy of progesterone-dependent breast cancers. The molecular mechanism by which antiprogestins repress progesterone receptor-regulated transcription are unknown, but they

could involve factors that block the activity of the basal transcriptional machinery.

To isolated possible antagonist-dependent interactors, distinct regions of the progesterone receptor hinge region (hge) and hormone binding domain (HBD) ($hPR_{637-933}$) were used to design polymerase chain reaction (PCR) primers (SEQ ID NOs:19-20). The PCT amplified $hPR_{637-933}$ sequence was cut with the appropriate restriction enzymes and cloned into BTM116 LexA fusion vector. Yeast cells transformed with the bait construct were treated with the antiprogestin RU486. Several cDNA sequences were obtained and sequenced (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17). The GeneBank database was searched for matching sequences.

Several of the cDNAs obtained by the methods described in Example 1 have not been previously isolated. One of these, termed TJ53 (SEQ ID NO:17) is a 3.2 kb clone encoding the interaction domain of a nuclear steroid receptor antagonist co-modulator. Its association with the HBD of the progesterone receptor is destabilized by the absence of hormone. TJ53 shares partial homology to the mouse nuclear receptor co-repressor protein found to specifically interact with the thyroid hormone/retinoic acid/vitamin D subfamily of nuclear receptors (Hörlein et al. (1995) Nature 377:397-403; Chen et al. (1995) Nature 377:454; Burris et al. (1995) Proc. Natl. Acad. Sci. 92:9525) (Fig. 2).

One of the isolated cDNAs was found to match the previously described ribosome protein L7 (SEQ ID NO:11) (Hemmerich et al. (1993) Nucleic Acid Res. 21:223). The evidence provided herein supports a new role for the L7 protein as a nuclear transcription protein. TJ21 (SEQ ID

NO:6) shares partial homology to the human Ku autoantigen p70 subunit (Chan et al. (1989) J. Biol. Chem. 264:3651).

The underlying mechanisms that render one ligand an antagonist, and another, often structurally very similar ligand, an agonist have not been known. It is also not known why some ligands have dual agonist/antagonist actions. The present invention is the first demonstration that recruitment of a cellular protein factor to the ligand/receptor complex is involved in distinguishing agonists from antagonists.

The invention provides nucleotide sequences encoding human nuclear co-modulator protein and peptide molecules that bind antagonist-occupied nuclear steroid receptors. These nucleotide sequences include DNA, cDNA, and RNA sequences which encode the hNRA-coM molecules of the invention. It is also understood that all nucleotide sequences encoding all or a portion of the hNRA-coM molecules of the invention are also included herein, as long as they encode a molecule with hNRA-coM activity, e.g., capable of binding an antagonist-occupied steroid receptor complex. Such nucleotide sequences include naturally occurring, synthetic, and intentionally manipulated nucleotide sequences. For example, hNRA-coM nucleotide sequences may be subjected to site-directed mutagenesis, which may result in dominant negative or dominant positive hNRA-coM mutant molecules. The nucleotide sequence for the hNRA-coM molecules of the invention also include antisense sequences. The nucleotide sequences of the invention further include sequences that are degenerate as a result of the genetic code.

The nucleotide sequences encoding the hNRA-coM molecules of the invention include SEQ ID NOs:1-18. A complementary sequence may include the antisense nucleotide. When the sequence is RNA, the

deoxynucleotides A, G, C, and T of the sequences shown are replaced by the ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-identified nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA or RNA that encodes hNRA-coM molecule under physiological conditions. Specifically, the fragments should hybridize to DNA or RNA encoding hNRA-coM molecule under stringent conditions, e.g., conditions which avoid non-specific binding (Sambrook et al. (1989) supra).

Minor modifications of the hNRA-coM molecule primary amino acid sequence may result in proteins and peptides which have substantially equivalent activity compared to the hNRA-coM molecules described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the proteins produced by these modifications are included herein. Further, deletions of one or more amino acids can also result in modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of small active molecules with may have a broader utility. For example, one may remove amino or carboxy terminal amino acids which are not required for hNRA-coM biological activity, or that enhance its activity in a dominant positive or dominant negative manner.

DNA sequences encoding hNRA-coM molecules can be expressed *in vitro* by DNA transfer into a suitable host cell. Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art.

The hNRA-coM molecules of the invention can also be used to produce antibodies which are immunoreactive or bind to epitopes of the hNRA-coM proteins and peptides.

Antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen-containing fragments of the protein by methods well known in the art.

Assays

Screening for compounds that have steroid antagonist activity. In one aspect, the present invention provides novel assay useful in identifying steroid receptor ligands having antagonist activities.

The hNRA-coM molecules of the invention are useful in identifying nuclear steroid receptor ligands which are steroid antagonists, as they bind to an antagonist-receptor complex but not to an agonist-receptor complex. As described in Example 2 below, the hNRA-coM molecule TJ53 (encoded by the nucleotide sequences SEQ ID NOs:17-18) binds a nuclear progesterone receptor only in the presence of the antiprogestin RU486, and does not bind an unliganded steroid receptor.

Although *in vitro* assays of the present invention can be configured in a number of ways, in a preferred configuration, a steroid receptor is contacted with a test ligand such that a receptor-ligand complex is formed, the complex is contacted with an hNRA-coM molecule, and the formation of a receptor-ligand-hNRA-coM complex is detected. Detection of the receptor-ligand-hNRA-coM complex may be accomplished in a number of ways. Binding of hNRA-coM molecule indicates that the bound ligand is a candidate antagonist. Non-binding of hNRA-coM molecule to the complex indicates that the bound ligand is not an antagonist, and may be a steroid agonist.

The method of the invention may be practiced in a variety of ways known to the art. For example, the assay components may be present in solution. Methods for determining optimal concentrations of each reagent are known in the art, as well as methods for optimizing conditions, e.g., temperature, incubation times, pH, etc., to provide a rapid and reproducible screening method. The binding of hNRA-coM to the receptor-ligand complex can be determined in variety of ways known to the art. For example, the hNRA-coM molecule may be labeled and the presence of label in the receptor-ligand complex determined. Further, interactions between hNRA-coM and steroid receptors can be measured by "pulldown" affinity chromatography between a resin-bound glutathione-S-transferase-steroid receptor fusion protein and a His-tagged hNRA-coM fusion protein that can be identified with antibodies having a fluorescent or a radioactive label. The ligand dependency of the interaction can be monitored by methods known to the art. Formation of a receptor-ligand-hNRA-coM complex is compared to complex binding in the presence of a steroid agonist (control). Preferably, binding of hNRA-coM to the receptor-ligand complex will be 50% or more greater when the ligand is an antagonist relative to an agonist; more preferably, hNRA-coM binding will be 75% or more greater to an antagonist-receptor complex relative to the agonist-receptor complex; most preferably, hNRA-coM binding will be 90% or more greater when the ligand is an antagonist. A candidate antagonist identified by the assay method of the invention can be tested in an animal model of steroid antagonist actions.

Useful also in the assay method of the invention are cells and animals in which the endogenous nucleotide sequence encoding the hNRA-coM molecule has been deleted and/or which are transfected with a nucleotide sequence

encoding a hNRA-coM molecule. Such cells and animals can be engineered by molecular recombinant and transgenic methods known in the art. Such cells and animals are useful for measurement of the transcriptional activity of a ligand-occupied steroid receptor in the presence or absence of recombinant hNRA-coM.

Therapeutic Uses

Enhancing steroid antagonist activity. The hNRA-coM molecules of the invention are able to bind to the steroid receptor-antagonist complex to mediate its biological activity. Although not bound by any theory, it is postulated that antagonist-bound receptor may bind either a co-repressor factor, such as that described by Hörlein et al. (1995) supra for TRV receptors, or a co-activator factor, such as those described by Halachmi et al. (1994) supra, Cavaillès et al. (1994) supra, and Baniahmad (1995) supra. Binding of the hNRA-coM molecules of the invention to the antagonist-receptor complex allows the antagonist to be biologically active and/or may be required for full antagonist activity. Binding of hNRA-coM may also block binding to the antagonist-occupied receptor complex by other co-activator factors which could inhibit or diminish antagonist activity. Thus, the hNRA-coM proteins of the invention enhance antagonist action.

The invention provides a therapeutically useful method for promoting the *in vivo* antagonist activity of an antagonist where it is clinically desirable by increasing the cellular levels of hNRA-coM. This may be achieved by providing a sense polynucleotide sequence (the DNA coding strand), a hNRA-coM molecule, or a hNRA-coM dominant positive mutant hNRA-coM molecule into the cell by methods known in the art. It can also include administration of a factor which enhances hNRA-coM gene

transcription. The method of the invention includes administration of a therapeutically effective amount of a hNRA-coM molecule to enhance steroid antagonist activity.

Therapeutic formulations of hNRA-coM molecules for treating antagonist-related conditions are prepared for storage by mixing one or more hNRA-coM molecules having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., 1980), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter ions such as sodium; and/or non-ionic surfactants such as Tween, Pluronics, or polyethylene glycol (PEG). hNRA-coM is also suitably linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. The amount of carrier used in a formulation may range from about 1 to 99%, preferably from about 80 to 99%, optimally between 90 and 99% by weight.

The hNRA-coM molecules to be used for *in vivo* administration must be sterile. This is readily accomplished by methods known in the art, for example, by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The hNRA-coM molecule ordinarily will be stored in lyophilized form or in solution.

Therapeutic hNRA-coM compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The administration of hNRA-coM may be in a chronic fashion using, for example, one of the following routes: injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, orally or using sustained-release systems as noted below. hNRA-coM is administered continuously by infusion or by periodic bolus injection if the clearance rate is sufficiently slow, or by administration into the blood stream or lymph. The preferred administration mode is targeted to the a specific tissue so as to direct the molecule to the source of antagonist action.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al. (1981) J. Biomed. Mater. Res. 15:167-277 and Langer (1982) Chem. Tech. 12:98-105, or poly(vinyl alcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al. (1983) Biopolymers 22:547-556), non-degradable ethylene-

vinyl acetate (Langer et al. (1981) supra), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

The hNRA-coM molecule also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate] microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release molecules for shorter time periods. When encapsulated molecules remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved, e.g., using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release hNRA-coM compositions also include liposomally entrapped hNRA-coM. Liposomes containing hNRA-coM are prepared by methods known *per se*: DE 3,218,121; Epstein et al. (1985) Proc. Natl. Acad. Sci. USA 82:3688-3692; Hwang et al. (1980) Proc. Natl. Acad. Sci. USA 77:4030-4034; EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type

in which the lipid content is greater than about 30 mol% cholesterol, the selected proportion being adjusted for the optimal hNRA-coM therapy. A specific example of a suitable sustained-release formulation is in EP 647,449.

An effective amount of hNRA-coM to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the clinician to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage of a hNRA-coM molecule used alone might range from about 1 μg/kg to up to 100 mg/kg of patient body weight or more per day, depending on the factors mentioned above, preferably about 10 μg/kg/day to 50 mg/kg/day.

Nucleotide sequences, including antisense sequences, can be therapeutically administered by various techniques known to those skilled in the art. Delivery of hNRA-coM nucleotide sequences can be achieved using free polynucleotide or a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of nucleotide sequences is the use of targeted liposomes.

Targeting of the therapeutic reagent to specific tissues is desirable to increase the efficiency of delivery. The targeting can be achieved by passive mechanisms via the route of administration. Active targeting to specific tissues can also be employed. The use of liposomes, colloidal suspensions, and viral vectors allows targeting to specific tissues by changing the composition of the formulation containing the therapeutic reagent, for example, by including molecules that act as receptors for components of the target tissues. Examples include sugars, glycoplipids, polynucleotides, or proteins. These molecules can be

included with the therapeutic reagent. Alternatively, these molecules can be included by indirect methods, for example, by inclusion of a polynucleotide that encodes the molecule, or by use of packaging systems that provide targeting molecules. Those skilled in the art will know, or will ascertain with the use of the teaching provided herein, which molecules and procedures will be useful for delivery of the therapeutic reagent to specific tissues.

Inhibition of steroid antagonist activity. As described above, the hNRA-coM molecules of the invention act as co-repressor factors which enhance the *in vivo* biological activity of steroid antagonists. Where it is desirable to suppress steroid antagonist activity *in vivo*, suppression can be achieved by administering a therapeutic reagent that inhibits hNRA-coM function or activity. Such therapeutic reagents can be used alone or in combination with other therapeutic reagents, for example, with chemotherapeutic agents in the treatment of malignancies.

The therapeutic reagents which may be employed are compounds which inhibit hNRA-coM expression, function, or activity, including nucleotides, polypeptides, and other molecules such as antisense oligonucleotides and ribozymes, and dominant negative mutants which can be made according to the invention and techniques known to the art. Dominant-negative forms of hNRA-coM which effectively displace or compete with hNRA-coM for substrate binding and/or phosphorylation can be used to decrease the inhibitory activity of receptor-antagonist complexes. Reagents which inhibit or decrease the expression of the endogenous hNRA-coM gene are also useful.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the compositions and perform the various methods of the present invention and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, parts are parts by weight, temperature is in degrees centigrade, and pressure is at or near atmospheric pressure. Efforts have been made to ensure accuracy with respect to numbers used, (e.g., length of DNA sequences, molecular weights, amounts, particular components, etc.) but some deviations should be accounted for.

Example 1. cDNA Sequences of Human Nuclear Steroid Receptor Antagonist Co-Modulator Proteins (hNRA-coM).

Plasmid construction. A lexA fusion vector, BTM116, (provided by Dr. S. Hollenberg, Fred Hutchinson Cancer Center, Seattle, WA) was digested with EcoRI and BamHI and dephosphorylated. The hinge region (hge) and contiguous hormone binding domain (HBD) of the human progesterone receptor (hPR) were amplified using the following oligonucleotide primers in a polymerase chain reaction (PCR): 5'-GCGGAATTCATGACTGAGCTGAAGGCAAAG (sense primer) (SEQ ID NO:19), and 5'-CCCAGATCTTCACTTTTTATGAAAGAGAAG (antisense primer) (SEQ ID NO:20). The primers contain restriction enzyme sites that allow in-frame cloning into the lexA fusion vector. The amplified hPR hgeHBD$_{637-933}$ DNA was digested with EcoRI and BamHI and ligated into the BTM116 vector. The BTM:hgeHBD vector was used as the "bait" plasmid in the yeast two-hybrid assay to (Fields & Song (1989) Nature 340:245-246).

Yeast strains. The L40 yeast strain (MATa, hisΔ300, trp1-901, leu2-3+112, ade2, LYS::$(lexAop)_4$-HIS3, URA3::$(lexAop)_8$-LacZ, GAL4, gal80) was used in the yeast two-hybrid assay (provided by Dr. S. Hollenberg).

Two hybrid library screen. A Gal4 activation domain/HeLa cDNA fusion library was purchased from Clontech Laboratories, Inc., and screened using the BTM:hgeHBD vector (Fig. 3). First, the BTM:hgeHBD construct was transformed into L40 cells using a the lithium acetate yeast transformation methods (Schiestl (1989) Curr. Genet. 16:339-346) to create L40:PR-hgeHBD. Then L40:PR-hgeHBD stain was transformed with the HeLa activation domain cDNA library. Eight million primary transformants were plated on -THULL plates (SD/-trp,-his,-ura,-leu,-lys) containing 1 μM RU486. His$^+$ colonies exhibiting β-galactosidase activity using the filter lift assay were further characterized. Electrocompetent HB101 *E. coli* were transformed with leu$^+$ library plasmids using a rapid yeast to bacteria electroporation protocol (Biotechniques (1993) 14:552). Positive controls were also performed on his$^+$ β-galactosidase$^+$ colonies obtained from the screen. For this, L40 was transformed with a) the positive library clones (autonomous activation control); b) BTM116 and the library clones (lexA interactor control); c) BTM:lamin and the library clones (nonspecific interaction control); and d) BTM:hgeHBD and the library clones (true positive control). For clone TJ53 (human N-coR), an additional control omitted hormone treatment. The unliganded BTM:hgeHBD failed to interact with the pGADGH:hNRA-coM. Only the RU486-occupied BTM:hgeHBD interacted with hNRA-coM.

Selection of cDNAs for further analysis. 131 his$^+$, β-gal$^+$ colonies were obtained and termed TJ1-TJ131 (Figs. 1A-1R). Of these, 22 size variants were selected for sequencing (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3,

SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18). The sequences obtained were compared to Genebank sequences. Several unique cDNA sequences were determined.

As shown in Fig. 2, the sequences of SEQ ID NO:17-18 encode a single protein having partially homology to the mouse nuclear receptor co-repressor protein that binds the thyroid hormone/retinoic acid/vitamin D subfamily of nuclear receptors (Hörlein et al. (1995) Nature 377:397-403; Chen et al. (1995) Nature 377:454; Burris et al. (1995) Proc. Natl. Acad. Sci. 92:9525).

One of the isolated cDNAs was found to match the previously described L7 translation regulatory protein (SEQ ID NO:11). L7 is a cytosolic ribosomal protein involved in translation. Thus, the evidence provided herein supports a new role for the L7 protein as a nuclear transcription protein. TJ21 (SEQ ID NO:6) shares partial homology to the human Ku autoantigen p70 subunit.

Example 2. Binding of hNRA-coM Molecule TJ53 to the Nuclear Steroid Receptor-Antagonist Complex.

The interaction of TJ53 (SEQ ID NO:17) with the nuclear steroid receptor was determined *in vivo* in yeast with two fusion proteins: LexA-DBD-PR HBD and the library fusion protein which is the Gal4 activation domain fused to the interaction domain of hNAR-coM. The interaction between these two fusion proteins was observed to occur only after treatment of yeast with the antiprogestin RU486, scored in a secondary screen by the presence of blue β-gal$^+$ cells previously selected for growth on histidine drop-out plates in the primary screen (Fields & Song (1989) Nature 340:245-246

Example 3. Therapeutic Use of hNRA-coM Molecule.

Tamoxifen, an antiestrogen, is tested in healthy women as a breast cancer preventative. Tamoxifen is expected to behave *in vivo* as an estrogen antagonist by inhibiting estrogen-stimulated growth of tumor tissue in the breast. In the uterus, however, tamoxifen acts like an estrogen agonist, e.g., by stimulating growth of endometrial cancers. Thus, it is desirable to maintain the antagonistic action of tamoxifen in the breast while eliminating its agonist action in the uterus. This is achieved by delivery of a hNRA-coM molecule to the uterus. Such local delivery can be achieved by introducing hNRA-coM encoding nucleotide sequences packaged into targeted liposomes into the uterus from an intrauterine devise inserted vaginally.

Example 4. Yeast Two-Hybrid Methods Using hNRA-coM Molecule for Identifying Steroid Antagonists.

The yeast two-hybrid system is used in a ligand screen. Yeast cells of the L40 genetic strain are engineered to contain plasmids expressing a fusion protein of LexA-DBD and the HBD region of the desired steroid receptor (e.g., the estrogen receptor) in conjunction with a plasmid expressing a fusion protein of the Gal4 activation domain fused to the interaction domain of hNRA-coM. The cells are then contacted with a candidate steroid receptor ligand. If the ligand is an antagonist (e.g., antiestrogen), its binding to the HBD of the steroid receptor will attract the interaction domain of hNRA-coM to the HBD through protein-protein contact. Such contact results in yeast cells which are able to grow on a histidine drop-out plate, and such cell colonies will turn blue due to expression of β-galactosidase activity.

Candidate ligands identified by the above described method as antagonists can be further assayed in a second screening method for partial agonist activity. For example, yeast cells are engineered by methods known in the art (see, for example, Guthrie & Fink (1991) Guide to Yeast Genetics and Molecular Biology, Methods Enzymol. Volume 194, Academic Press, San Diego, CA) so that the endogenous hNRA-coM gene is deleted. This eliminates effects in the cells due to antagonist activity of a ligand. The cells are then engineered to express a plasmid encoding the desired steroid receptor (e.g., estrogen receptor), and a plasmid expressing a promoter containing the appropriate response element (e.g., estrogen response element) fused to a reporter gene, for example, β-galactosidase. The cells are contacted with the candidate ligand previously identified in the first screen as an antagonist (e.g., antiestrogen). If the ligand stimulates β-galactosidase activity, the candidate ligand has partial agonist activity. If the desired candidate ligand is a "pure" antagonist, a ligand having partial agonist activity would be discarded. Thus, the second screening method can be used to select "pure" antagonists from among ligands having mixed antagonist/agonist activities.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE UNIVERSITY OF COLORADO

(ii) TITLE OF INVENTION: STEROID RECEPTOR ANTAGONIST CO-MODULATOR AND METHODS FOR USING SAME

(iii) NUMBER OF SEQUENCES: 20

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: AscIII

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 440 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: position 5, 22, 82,115, 165, 217, 236, 298, 304, 340, 334, 337, 341, 351, 362, 370, 383, 427, 433
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGNAGATC CCAACAAAAG ANCTGACTGT GTCCCGGTGA GATCGGAGAT CGACCGTCTA 60
CAAGCTTCGG GTGATCGGGG CNGTGCTAGG TAAGGGAGTG AGAGAAGGAC AAGCNGGCAG 120
AGTTGCAGGG ATCACGTGTA AAGCGCTGTC AGTATCGGGC ATGTNTATTC TTGGTCTATC 180
CGAATTGCAA GACTGGAGTG GATGGGGATG AGAGGCNGTT GCCAGGACTT TCCATNGCCG 240
GCGAGAGTGA GCTGCGTCTC AGATCACAGT TGGTTACGTC ACAAAGTGCA GTGCAAGNCG 300
GTTNCATAGC CAGTAGCAGA TACTGCATCN TGCNTCNTAC NGAGCCTAGT NTAGCGGACG 360
TNATTACGGN NGGAAGGTTC AGNCTTGACT ATGCCAATAC GTGTAAAAAA AATGGGGCGC 420
ACCTTTNTGT ATNTGCCTAG 440

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 487 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 2, 20, 236, 241, 262, 280, 417, 421, 423, 438, 464, 469.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TNTCGATGAT GAGTACCACN AACCAAAAAA AGAGTCCTAG AACTAGTGGA TCCCCGGGCT 60
GCAGGAATTC GGCACGAGCT TACCTGACCT TTGATGCCGA CGTGAAGGAA GAGTTTGTGG 120
AGGATGCGGC TGCTCTGAAA GCTCTGTTCC AGCTCAGCAG GTTGGAGGAG AGGTCAGTGC 180
TCTTTGCGGT GGCTCAGCGC TGGTGAACTG CAACAACAGG TATNAATTAC GAGGANGCCN 240
NCCCCAAGAT GGTGGGAGCT GGNCAAGTAT GCCAAGCAGN ATTGCCCGAG CAGCACCCAA 300
GGACAAGCCA AGCTTCGTGC GGGCTCGGGT GAAGAAGCTG CTGGCAGCGG GTGTGGTGTC 360
GGCATGGTGT GCATGGTGAA GACGGAGAGC CTGTGCTGAC CAGTTCCTGC AGAGAGNTGT 420
CTNCAGGGTC TTCTTGGNTT TAGTGGAAGA GTAGAGGACC AAGNATTTNG GTGCCAGGGA 480
GGGGGAA 487

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 458 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 6, 73, 83, 99, 101, 113, 127, 185, 382, 414, 437, 441
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACCGGNGATC CTCTAGAGTC GACCTGCAGG CATGCAAGCT TGTTTATTCT ATCGTGTCAC 60
CTAACTAGCT TGNCGTAATC ATNGCCATAG TTGTTTCCNG NGTGATATAG TTNTCCGCAT 120
CACATTNCCA CACAACATAC GAGCCGGAAG CATAATGTGT AAAGCCTGGG GTGCCTAATG 180
AGTCNAGCTA ACTCACATAG AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC 240
TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG 300
GGAGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGATCGG TTGCGGCGAG 360
CGGTATCAGC TCACTCAAGG GNGGTATACG GGTATCCACA GAATCAGGGG ATANCGCAGG 420
AAAGACATGT GAGCAANAGG NCAGCACAAG GCCAGGAA 458

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 204 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 7, 199
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCTTTNTAA TATTATCGAT GATGAGATAC CACAAACCAA AAAAAGAGAC CTAGAACTAG 60
TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAGCTTACCT GACCTCTGAT GCCGACGTGA 120
AGGAAGAGTT TGTGGAGGAT GCGGCTGCTC TGAAAGCTCT GCTCCAGCTC AGCAGGTTGG 180
AGGAGAGGTC AGTGCTCTNT GCGG 204

(2) INFORMATION FOR SEQ ID NO:5
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 32, 177
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATACCCACC AAACCAAAAA AAGAGACCTA GNAACTAGTG GATCCCCCGG GCTGCAGGAA 60
TTCGGCACGA GCTTACCTGA CCTTTGATGC CGACGTGAAG GAAGAGTTTG TGGAGGATGC 120
GGCTGCTCTG AAAGCTCTGT TCCAGCTCAG CAGGTTGGAG GAGAGGTCAG TGCTCTN 177

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 1, 54, 108, 126, 134, 148, 151, 198, 228, 243, 250, 278, 293, 355, 358, 359, 370, 390
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

NTTTTTACTA TTTTCGATGA TGAGATACCC ACAAACCAAA AAAAGAGACC TAGNAACTAG 60
TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAGCGGCACG AGAACGGNTT GATGATCCAG 120
GTTTGNTGCT CATNGCNTTT CAAGACGNTG NTACTGCTGA AGAAACAACA TTACCTGAGG 180
GCCTCCCTAT TCGTGTANCC AGAGGTAGTC GGTTGGTGAT TGGGGAGNTC AANCCTGTTC 240
AGNGCTCTGN TCATCAAGTG TCTGGAGAAG GAGGTTGNAG CATTGTGCAG ATNCACACCC 300
CGCAGGAACA TCCCTCCTTA TTTGTGGGTT TGGGTGCACA GAAAGAAGAG TTGGNTGNNC 360
AGAAAATCAN GTGATCCTCC AGGCTCCAGN TGGTCTTTTT ACCCTTGGGT GATGATAAAA 420

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 17, 71, 78, 81, 83, 122, 129, 134, 147, 154, 164, 199, 209, 224, 227, 237, 240, 289.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TATTTATCGA TGATGANGTT ACCCACCAAA CCAAAAAAAG AGATCCTAGA AACTAGTGGG 60
ATCCCCCGGG NTGTATANTA NTNTCGATGA TGATGATACC ACACAGCCAC TATAAGAGAC 120
CNAGCAGTNG TGGNTCCCCC GGGCTGNAGG AATNCGGGAC GATNTCGTGC CGCGGTCCGA 180
GCACATTAGA GGTTATCANA AGTCCACTNA AACTGCTGAT TCGNAANCTT CCCTTCNAGN 240
GTCTGGTGAG GAGAAATTGC TCAGCGACTT CCCACAGGTC TTGGTGTCNC AGAG 294

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 55, 65, 81, 99, 105, 122, 136, 137, 144, 161, 178, 188, 199, 200, 202, 207, 211, 212, 217, 231
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTTAATATC TATCGATGAT GAAGATACCC ACCAAACCAA AAAAAGAGAC CTAGNAACTA 60
GTGGNATCCC CCGGGCTGTA NGAATCCGGC ACGATGATNG TACCNCTCAG CTCTATGGAG 120
GNCGAGCAGT TGATANNCCC GAANTGAAGA AATACGAAGA NGATATCGAC GGCTTGTNTG 180
ATCCAGGNTT GATGTTCANN GNTCACNAAG NNGCTGNTTC CTAATGTACA NTACACCATT 240

CT 242

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 22, 77, 78, 79, 83, 84, 163, 168, 204, 209, 237, 278, 281, 249, 405
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTTACTATT ATCGATGATG ANGATACCAC AAACCAAAAA AAGAGACCTA GAAACTAGTG 60
GGATCCCCCG GGCTGCNNNA TTNNGGCACG ATGACTATCA TATAGTAAAA CCAGGCCATG 120
ACCCCTAACA GGGGCCCTCT CAGGCCTCCT AATGACCTCC GGNCTAGNCA TGTGATTTCA 180
CTTCCACTCC ATAACGGTCC TCATACTAGG GCTNCTAANC CAACACACTA ACCATATNCC 240
AATGATGGCG CGATGTTACA CGAGAAAGCA CATACCANGG NCACCACACA CCACCTGTCC 300
AAAAAGGGCT TCGATATGGG ATAATCCTAT TTATTACCTC AGAAGTTTNT TTCTTCGCAG 360
GATGTTCTGA GGCTTTTACA CTCCAGGCTA GCCCCTACCC CCCANCTAGG AGGGCACTGG 420

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 422 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 7, 117, 120, 191, 194, 198, 212, 213, 258, 268, 281, 286, 297, 304, 335, 347, 362, 365, 384, 387, 402
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TATTATNCGA TGATGAGCTA CCCACAAACC AAAAAAAGAG ACCTAGAATA GTGGATCCCC 60
CGGGCTGCAG GAATTCGGCA CGAGGCCAGC CCCAGGGCCC TGGGCTTCGA ACCCGGNTGN 120
TGATCACAGG CCTGTTCGGG GCTGGACTCG GTGGAGCCTG GCTGGCCCTG AGGGCTGAGA 180
AGGAGAGGCT NTANCAGNAA ATGTGAACAG ANNCCATGCG CCAGGCAGCT GTGGGCCAGG 240
GCGAATTCCA CCTGCTGNAT CACAGAGGNC GGGATCGCTG NAAGGNTGAC TGCCAGNGAC 300
AGTNGGTGCT GATGTACTTG GGCTTCACTC ACTGNCCTGA CATCTGNCCA GACGAGCTGG 360
ANAANCTCAT GCAGGTGGTG CGGNAGNTAG AAGCAGAGCC TNGTTGGCTC CAGTGCAGGC 420
TG 422

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 25, 72, 148, 168, 179, 197, 245, 255, 279, 298, 329
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCCACAAAC CAAAAAAAGA GACCNAGAAT AGTGGATCCC CCGGGCTGCA GGAATTCGGC 60
ACGAGGGGTG TNGAAGAGAA GAAGAAGGAG GTTCCTGCTG TGCCAGAAAC CCTTAAGAAA 120
AAGCGAAGGA ATTTCGCAGA GCTGAAGNAT CAAGCGCCTG AGAAAGANGT TTGGCCAANA 180
GATGCTTCGC AAGGCANGGA GGAAGCTTAT CTATGAAAAA GCAAAGCACT ATCACAAGGA 240
ATATNGGCAG ATGTNCAGAA CTGAAATTCG AATGGCGANG ATGGCAAGAA AAGCTGGNAA 300
TTCTATGTAC CTGCAGAACC CAAATTGGNG TTGTCATCAG AATCAGAGGT 350

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 1, 2, 4, 7, 12, 18, 21, 24, 33, 38, 39, 41, 46, 50, 51, 53, 57, 61, 70, 71, 74, 77, 79, 81, 89, 92, 93, 99, 101, 104, 105, 106, 107, 109, 112, 115, 116, 118, 125, 126, 128, 132, 135, 136, 141, 143, 147, 153, 154, 158, 165, 166, 168, 172, 173, 175, 179, 180, 181, 182, 183, 186, 189, 190, 191, 194, 196, 201, 210, 214, 215, 219, 224, 237, 238, 240, 246, 252, 253, 254, 260, 262, 268, 272, 274, 276, 281, 287, 288, 290, 291, 296, 297, 300
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
NNCNGGNTGA AGGANTTNGT NCANTGATGA GCNCTCGNNC NAAACNAAGN NGNGAGNCCC   60
NGAACTAGTN NTTNTTNTNG NCTACAGANA ANNCCGAANG NGANNNNGNA GNGGNNGNCA  120
ACCANNCNCT GNGANNCTGG NGNGGGNACT GTNNGACNAG GGGGNNGNGC ANNGNAGGNN  180
NNNGANCTNN NACNCNGCTC NGCGACTCCN ACANNGCANT ATTNGGAGCT GCTCAGNNCN  240
TGAAANCTGA TNNNCGCCTN GNGAAGTNTA TNTNGNAACT NCTCTTNNTN NCAGNNCCCN  300
```

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 8, 76, 87, 207, 235, 246
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
TTTTATANTA TCGATGATGA GATACCCACA AACCAAAAAA AGAGACCTAG AAACTAGTGG   60
TATCCCCCGG GCTGCNGGAA TTCGGCNCGA GGGTCCTCGA GGTGCCTCCT GTTGTGTATT  120
CCCGGCAGGA GCAGGAGGAG GAGGGCCGGA AGCGGTATGA AGCCCAGAAG CTGGAGCGCA  180
TGGAGACCAA GTGGAGGAAC GGGGGANATC GTCCAGCCAG TCCTCAACCC AGAGNCGAAC  240
ACTGTCAGNT ACAGNCAGTC CAGCTTGATC CACCTGGTGG GGGCTTCAGA CTGCACCCTG  300
CACGGNTTTG TGCACGGAGG TGTGA                                        325
```

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 2, 204, 283, 288, 338
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
TNTACTATTA TTCGATGATG ATGATACCCA CAAACCAAAA AAAGAGACCT AGAAATAGTG   60
GATCCCCCGG GCTGCAGGAA TTCGGCACGA GGCAGTGTAT GGGGGCAGCT ACCACTCTAT  120
GAGCTCGATG GCACGAGCAG CGTTCTCTGA GGATGGGGGC CTGATGGATG GTGGCATGGA  180
CCTCAACATG GAGCAGGGCA TTGNAGAGCA CCTTAAGGAT GTGATCCTAC TGACAGGCAT  240
CGTGCAGGTG CTCAGCTGCT TCTCTCTCTA TGTCTGGTCC TTNTGGGNTT CTGGCTCCAG  300
GCCGGGCCCT TTACCTCCTG TGGGTGAATG TGCTGGGNC                          339
```

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 250 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 76, 85, 102, 105, 106, 111, 115, 116, 117, 118, 123, 147, 150, 162, 169, 174, 182, 195, 207, 208, 211, 221, 226, 235, 241, 242
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGATGATGAG TTACCCACCA AACCAAAAAA AGAGATCCTA GAAACTAGTG GGATCCCCCG 60
GGCTGTAGGA ATTCGNGACG AGAGNAGCTC TCCTGCCACA GNTCNNCATC NCCTNNNNGA 120
TCNTCGCCTT GTAGCAATTC GGTACTNCAN TCACTCATCG GNACAGGANT AGCNCTACAG 180
CNGTCTGAGA CATCNGATGA TATCAANNGT NCACTGAACT NCTGCTNCGG AACTNCCAGA 240
NNAGAGTCTC 250

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown
(B) LOCATION: Positions 22, 97, 130, 132, 143, 148, 151, 152, 156, 165, 166, 170, 186, 195, 206, 211, 214, 220, 238, 249, 251, 264, 276, 283, 287, 289, 290, 293, 302, 303, 306, 311, 316, 319, 326, 330, 338, 348, 369, 370, 374, 375, 376, 377, 378, 379, 380, 381, 431
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTTTTACTAT TATCGATGAT GNAGTACCCA CCAAACCAAA AAAAGAGATC CTAGAAACTA 60
GTGGGATCCC CCGGGCTGCT GGAATTCGGC TCGAGGNCTC CTAAAAAAGG GCAAAGCTCA 120
AGTCTNGAAN ANTGGAGAAG GANGTAANGA NNTTTNAACA AAACCNNCCN GCCCAAGGAA 180
AAGGGNAAAA AAGGNGATTC ATCAGNGAAC NCTNTTGGGN AGGACCCCGG TTTAACANTT 240
TTTTAAGANG NGAAGTTTTG GCCNTGGGCC AAAAANAAGT TGNGTTNCNN GANAAAATGC 300
CNNCCNGGAG NGCCTNACNA TGGAANGAAN AAAAAAGNTT TGGGGTTNCC TTGGGGATTG 360
ATGAAAAANN AACNNNNNNN NAAATGGTTG GTCAAGAGGA TGTTGAACCC TTGGTCCCCC 420
CACAAAACCA NT 432

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 971 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown, M is A or C, S is C or G, K is G or T/U
(B) LOCATION: Positions 343, 356, 384, 421, 514
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATATGTTC GCTCCCCTTC CCCTTCAGTA AGAACTCAGG AGACAATGTT GCAACAGAGA 60
CCCAGTGTTT TCCAAGGAAC CAATGGAACC AGTGTAATCA CACCTTTGGA TCCAACTGCT 120
CAGCTACGAA TCATGCCAAT GCCTGCTGGG GGGCCTTCAA TAAGCCAAGG CCTGCCAGCC 180
TCCCGTTACA ACACTGCTGC GGATGCCCTG GCTGCTCTTG TGGATGCTGC AGCTTCTGCA 240
CCCCAGATGG ATGTGTCCAA AACAAAAGAG AGTAAGCATG AAGCTGCCAG GTTAGAAGAA 300
AATTTGAGAA GCAGGTCAGC AGCAGTTAGT GAACAGCAGC AGSTAGAGCA GAARAMCCTG 360
GAGGTGGAGA AGAGATCTGT TCAKTGTCTA TACACTTCTT CAGCCTTTCC AAGTGGCAAG 420
SCCCAGCCTC ATTCTTCAGT AGTTTATTCT GAGGCTGGGA AAGATAAAGG GCCTCCTCCA 480
AAATCCAGAT ATGAGGAAGA GCTAAGGACC AGASGGAAGA CTACCATTAC TGCAGCTAAC 540
TTCATAGACG TGATCATCAC CCGGCAAATT GCCTCGGACA AGGATGCGAG GGAACGTGGC 600
TCTCAAAGTT CAGACTCTTC TAGTAGCTTA TCTTCTCACA GGTATGAAAC ACCTATCGAT 660
GCTATTGAGG TGATAAGTCC TGCCAGCTCA CCTGCGCCAC CCCAGGAGAA ACTGCAGACC 720
TATCACCCAG ATGTTGTTAA AAGGCAAATC AAGCGGAAAA TGATCCTACC AGACAATATG 780
AAGGACCATT ACATCACTAT CGACCACAGC AGGAATCACC ATCCCCAACA ACAGCTGCCC 840

CCTTCTTCAC AGGCAGAGGG AATGGGGCAA GTGCCAGGAC CCATCGGCTG ATCACACTTG 900
CTGATCACAT CTGTCAAATT ATCACACAAG ATTTGCTAGA AATCAAGTTT TCCGTAGATC 960
AGCTCCCCAG T 971

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: N is unknown, Y is C or T/U
(B) LOCATION: Positions 127, 128, 283, 284, 285, 304, 305, 306
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
CCCATCTCCC CACCCCAGGT TCCGGGTGTG CATGAGAAAC AGGACAGCTT GCTGCTCTTG 60
TCTCAGAGGG GCGCAGAGCC TGCTGAGCAG AGGAATGATG CCCGCTCACC AGGCAGTATA 120
AGCTACYYGC CTTCATTCTT CACCAAGCTT GAAAGTACAT CACCCATGGT TAAATCTAAG 180
AAACCTGAAA TTTTTCCCAA GTGGAACTCC TCTGTTGGAG GTGACTCTGA TATGGCATCT 240
GCTCACCCAG GAACTGAGAT CTTCAATCTG CCACCAGTTA CTNNNTCTGG CGCAGTCACC 300
TCTNNANGCC ATTCTTTTGC TGATCCCGCC AGTAACCTTG GGCTGGA 347

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GCGGAATTCA TGACTGAGCT GAAGGCAAAG 30

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
CCCAGATCTT CACTTTTTAT GAAAGAGAAG 30

What is claimed is:

1. Isolated DNA encoding a human nuclear steroid hormone receptor antagonist co-modulator molecule (hNRA-coM).

2. The isolated DNA of claim 1, having a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or degenerate variants thereof.

3. Substantially pure hNRA-coM molecule comprising an amino acid sequence encoded by any one of the DNA sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18, and capable of binding an antagonist-steroid receptor complex.

4. A recombinant expression vector containing the nucleotide sequence of claim 2.

5. A host cell comprising the nucleotide sequence of claim 2.

6. A purified antibody which binds specifically to the molecule of claim 3.

7. An assay method, comprising:

a) contacting a steroid receptor with a test ligand, wherein a receptor-ligand complex is formed;

b) contacting an hNRA-coM molecule with the receptor-ligand complex;

c) determining binding of hNRA-coM molecule to the receptor-ligand complex, wherein binding of said hNRA-coM molecule to said complex indicates that the ligand is a steroid receptor antagonist.

8. A method for inhibiting steroid antagonist activity, comprising administering a therapeutic agent which inhibits endogenous expression of hNRA-coM molecule.

9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier admixed with a therapeutically effective amount of a hNRA-coM molecule.

10. A method for enhancing steroid antagonist activity, comprising increasing the cellular level of hNRA-coM molecule.

TJ1

TTCGNAGATC CCAACAAAAG ANCTGACTGT GTCCCGGTGA GATCGGAGAT CGACCGTCTA
CAAGCTTCGG GTGATCGGGG CNGTGCTAGG TAAGGGAGTG AGAGAAGGAC AAGCNGGCAG
AGTTGCAGGG ATCACGTGTA AAGCGCTGTC AGTATCGGGC ATGTNTATTC TTGGTCTATC
CGAATTGCAA GACTGGAGTG GATGGGGATG AGAGGCNGTT GCCAGGACTT TCCATNGCCG
GCGAGAGTGA GCTGCGTCTC AGATCACAGT TGGTTACGTC ACAAAGTGCA GTGCAAGNCG
GTTNCATAGC CAGTAGCAGA TACTGCATCN TGCNTCNTAC NGAGCCTAGT NTAGCGGACG
TNATTACGGN NGGAAGGTTC AGNCTTGACT ATGCCAATAC GTGTAAAAAA AATGGGGCGC
ACCTTTNTGT ATNTGCCTAG

FIG. 1A

TJ2

TNTCGATGAT GAGTACCACN AACCAAAAAA AGAGTCCTAG AACTAGTGGA TCCCCGGGCT
GCAGGAATTC GGCACGAGCT TACCTGACCT TTGATGCCGA CGTGAAGGAA GAGTTTGTGG
AGGATGCGGC TGCTCTGAAA GCTCTGTTCC AGCTCAGCAG GTTGGAGGAG AGGTCAGTGC
TCTTTGCGGT GGCTCAGCGC TGGTGAACTG CAACAACAGG TATNAATTAC GAGGANGCCN
NCCCCAAGAT GGTGGGAGCT GGNCAAGTAT GCCAAGCAGN ATTGCCCGAG CAGCACCCAA
GGACAAGCCA AGCTTCGTGC GGGCTCGGGT GAAGAAGCTG CTGGCAGCGG GTGTGGTGTC
GGCATGGTGT GCATGGTGAA GACGGAGAGC CTGTGCTGAC CAGTTCCTGC AGAGAGNTGT
CTNCAGGGTC TTCTTGGNTT TAGTGGAAGA GTAGAGGACC AAGNATTTNG GTGCCAGGGA
GGGGGAA

FIG. 1B

TJ11-L1 base 43-500

ACCGGNGATC CTCTAGAGTC GACCTGCAGG CATGCAAGCT TGTTTATTCT ATCGTGTCAC
CTAACTAGCT TGNCGTAATC ATNGCCATAG TTGTTTCCNG NGTGATATAG TTNTCCGCAT
CACATTNCCA CACAACATAC GAGCCGGAAG CATAATGTGT AAAGCCTGGG GTGCCTAATG
AGTCNAGCTA ACTCACATAG AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC
TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG
GGAGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGATCGG TTGCGGCGAG
CGGTATCAGC TCACTCAAGG GNGGTATACG GGTATCCACA GAATCAGGGG ATANCGCAGG
AAAGACATGT GAGCAANAGG NCAGCACAAG GCCAGGAA

FIG. 1C

TJ15-L1 base 85-288

TGCTTTNTAA TATTATCGAT GATGAGATAC CACAAACCAA AAAAAGAGAC CTAGAACTAG
TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAGCTTACCT GACCTCTGAT GCCGACGTGA
AGGAAGAGTT TGTGGAGGAT GCGGCTGCTC TGAAAGCTCT GCTCCAGCTC AGCAGGTTGG
AGGAGAGGTC AGTGCTCTNT GCGG

FIG. 1D

TJ16-L1 base 22-198

GATACCCACC AAACCAAAAA AAGAGACCTA GNAACTAGTG GATCCCCCGG GCTGCAGGAA
TTCGGCACGA GCTTACCTGA CCTTTGATGC CGACGTGAAG GAAGAGTTTG TGGAGGATGC
GGCTGCTCTG AAAGCTCTGT TCCAGCTCAG CAGGTTGGAG GAGAGGTCAG TGCTCTN

FIG. 1E

TJ21-L1 base 9-428

NTTTTTACTA TTTTCGATGA TGAGATACCC ACAAACCAAA AAAAGAGACC TAGNAACTAG
TGGATCCCCC GGGCTGCAGG AATTCGGCAC GAGCGGCACG AGAACGGNTT GATGATCCAG
GTTTGNTGCT CATNGCNTTT CAAGACGNTG NTACTGCTGA AGAAACAACA TTACCTGAGG
GCCTCCCTAT TCGTGTANCC AGAGGTAGTC GGTTGGTGAT TGGGGAGNTC AANCCTGTTC
AGNGCTCTGN TCATCAAGTG TCTGGAGAAG GAGGTTGNAG CATTGTGCAG ATNCACACCC
CGCAGGAACA TCCCTCCTTA TTTGTGGGTT TGGGTGCACA GAAAGAAGAG TTGGNTGNNC
AGAAAATCAN GTGATCCTCC AGGCTCCAGN TGGTCTTTTT ACCCTTGGGT GATGATAAAA

FIG. 1F

TJ25-L1 base 18-311

TATTTATCGA TGATGANGTT ACCCACCAAA CCAAAAAAAG AGATCCTAGA AACTAGTGGG
ATCCCCCGGG NTGTATANTA NTNTCGATGA TGATGATACC ACACAGCCAC TATAAGAGAC
CNAGCAGTNG TGGNTCCCCC GGGCTGNAGG AATNCGGGAC GATNTCGTGC CGCGGTCCGA
GCACATTAGA GGTTATCANA AGTCCACTNA AACTGCTGAT TCGNAANCTT CCCTTCNAGN
GTCTGGTGAG GAGAAATTGC TCAGCGACTT CCCACAGGTC TTGGTGTCNC AGAG

FIG. 1G

TJ30-L1 base 8-250

TTTTAATATC TATCGATGAT GAAGATACCC ACCAAACCAA AAAAAGAGAC CTAGNAACTA
GTGGNATCCC CCGGGCTGTA NGAATCCGGC ACGATGATNG TACCNCTCAG CTCTATGGAG
GNCGAGCAGT TGATANNCCC GAANTGAAGA AATACGAAGA NGATATCGAC GGCTTGTNTG
ATCCAGGNTT GATGTTCANN GNTCACNAAG NNGCTGNTTC CTAATGTACA NTACACCATT
CT

FIG. 1H

TJ39-L1 base 7-426

TTTTACTATT ATCGATGATG ANGATACCAC AAACCAAAAA AAGAGACCTA GAAACTAGTG
GGATCCCCCG GGCTGCNNNA TTNNGGCACG ATGACTATCA TATAGTAAAA CCAGGCCATG
ACCCCTAACA GGGGCCCTCT CAGGCCTCCT AATGACCTCC GGNCTAGNCA TGTGATTTCA
CTTCCACTCC ATAACGGTCC TCATACTAGG GCTNCTAANC CAACACACTA ACCATATNCC
AATGATGGCG CGATGTTACA CGAGAAAGCA CATACCANGG NCACCACACA CCACCTGTCC
AAAAAGGGCT TCGATATGGG ATAATCCTAT TTATTACCTC AGAAGTTTNT TTCTTCGCAG
GATGTTCTGA GGCTTTTACA CTCCAGGCTA GCCCCTACCC CCCANCTAGG AGGGCACTGG

FIG. 1I

TJ42-L1 base 79-500

TATTATNCGA TGATGAGCTA CCCACAAACC AAAAAAAGAG ACCTAGAATA GTGGATCCCC
CGGGCTGCAG GAATTCGGCA CGAGGCCAGC CCCAGGGCCC TGGGCTTCGA ACCCGGNTGN
TGATCACAGG CCTGTTCGGG GCTGGACTCG GTGGAGCCTG GCTGGCCCTG AGGGCTGAGA
AGGAGAGGCT NTANCAGNAA ATGTGAACAG ANNCCATGCG CCAGGCAGCT GTGGGCCAGG
GCGAATTCCA CCTGCTGNAT CACAGAGGNC GGGATCGCTG NAAGGNTGAC TGCCAGNGAC
AGTNGGTGCT GATGTACTTG GGCTTCACTC ACTGNCCTGA CATCTGNCCA GACGAGCTGG
ANAANCTCAT GCAGGTGGTG CGGNAGNTAG AAGCAGAGCC TNGTTGGCTC CAGTGCAGGC

FIG. 1J

TJ48-L1 base 32-381

ACCCACAAAC CAAAAAAAGA GACCNAGAAT AGTGGATCCC CCGGGCTGCA GGAATTCGGC
ACGAGGGGTG TNGAAGAGAA GAAGAAGGAG GTTCCTGCTG TGCCAGAAAC CCTTAAGAAA
AAGCGAAGGA ATTTCGCAGA GCTGAAGNAT CAAGCGCCTG AGAAAGANGT TTGGCCAANA
GATGCTTCGC AAGGCANGGA GGAAGCTTAT CTATGAAAAA GCAAAGCACT ATCACAAGGA
ATATNGGCAG ATGTNCAGAA CTGAAATTCG AATGGCGANG ATGGCAAGAA AAGCTGGNAA
TTCTATGTAC CTGCAGAACC CAAATTGGNG TTGTCATCAG AATCAGAGGT

FIG. 1K

TJ49-L1 base 1-300

NNCNGGNTGA AGGANTTNGT NCANTGATGA GCNCTCGNNC NAAACNAAGN NGNGAGNCCC
NGAACTAGTN NTTNTTNTNG NCTACAGANA ANNCCGAANG NGANNNNGNA GNGGNNGNCA
ACCANNCNCT GNGANNCTGG NGNGGGNACT GTNNGACNAG GGGGNNGNGC ANNGNAGGNN
NNNGANCTNN NACNCNGCTC NGCGACTCCN ACANNGCANT ATTNGGAGCT GCTCAGNNCN
TGAAANCTGA TNNNCGCCTN GNGAAGTNTA TNTNGNAACT NCTCTTNNTN NCAGNNCCCN

FIG. 1L

TJ54-L1 base 6-330

TTTTATANTA TCGATGATGA GATACCCACA AACCAAAAAA AGAGACCTAG AAACTAGTGG
TATCCCCCGG GCTGCNGGAA TTCGGCNCGA GGGTCCTCGA GGTGCCTCCT GTTGTGTATT
CCCGGCAGGA GCAGGAGGAG GAGGGCCGGA AGCGGTATGA AGCCCAGAAG CTGGAGCGCA
TGGAGACCAA GTGGAGGAAC GGGGGANATC GTCCAGCCAG TCCTCAACCC AGAGNCGAAC
ACTGTCAGNT ACAGNCAGTC CAGCTTGATC CACCTGGTGG GGGCTTCAGA CTGCACCCTG
CACGGNTTTG TGCACGGAGG TGTGA

FIG. 1M

TJ57-L1 base 2-340

TNTACTATTA TTCGATGATG ATGATACCCA CAAACCAAAA AAAGAGACCT AGAAATAGTG
GATCCCCCGG GCTGCAGGAA TTCGGCACGA GGCAGTGTAT GGGGGCAGCT ACCACTCTAT
GAGCTCGATG GCACGAGCAG CGTTCTCTGA GGATGGGGGC CTGATGGATG GTGGCATGGA
CCTCAACATG GAGCAGGGCA TTGNAGAGCA CCTTAAGGAT GTGATCCTAC TGACAGGCAT
CGTGCAGGTG CTCAGCTGCT TCTCTCTCTA TGTCTGGTCC TTNTGGGNTT CTGGCTCCAG
GCCGGGCCCT TTACCTCCTG TGGGTGAATG TGCTGGGNC

FIG. 1N

TJ108-L1 base 1-250

CGATGATGAG TTACCCACCA AACCAAAAAA AGAGATCCTA GAAACTAGTG GGATCCCCCG
GGCTGTAGGA ATTCGNGACG AGAGNAGCTC TCCTGCCACA GNTCNNCATC NCCTNNNNGA
TCNTCGCCTT GTAGCAATTC GGTACTNCAN TCACTCATCG GNACAGGANT AGCNCTACAG
CNGTCTGAGA CATCNGATGA TATCAANNGT NCACTGAACT NCTGCTNCGG AACTNCCAGA
NNAGAGTCTC

FIG. 1O

TJ112-L1 base 10-433
TTTTTACTAT TATCGATGAT GNAGTACCCA CCAAACCAAA AAAAGAGATC CTAGAAACTA
GTGGGATCCC CCGGGCTGCT GGAATTCGGC TCGAGGNCTC CTAAAAAAGG GCAAAGCTCA
AGTCTNGAAN ANTGGAGAAG GANGTAANGA NNTTTNAACA AAACCNNCCN GCCCAAGGAA
AAGGGNAAAA AAGGNGATTC ATCAGNGAAC NCTNTTGGGN AGGACCCCGG TTTAACANTT
TTTTAAGANG NGAAGTTTTG GCCNTGGGCC AAAAANAAGT TGNGTTNCNN GANAAAATGC
CNNCCNGGAG NGCCTNACNA TGGAANGAAN AAAAAAGNTT TGGGGTTNCC TTGGGGATTG
ATGAAAAANN AAC******* *AAATGGTTG GTCAAGAGGA TGTTGAACCC TTGGTCCCCC
CACAAAACCA NT

FIG. 1P

nucleotide 5418--
GGATATGTTC GCTCCCCTTC CCCTTCAGTA AGAACTCAGG AGACAATGTT GCAACAGAGA
CCCAGTGTTT TCCAAGGAAC CAATGGAACC AGTGTAATCA CACCTTTGGA TCCAACTGCT
CAGCTACGAA TCATGCCAAT GCCTGCTGGG GGGCCTTCAA TAAGCCAAGG CCTGCCAGCC
TCCCGTTACA ACACTGCTGC GGATGCCCTG GCTGCTCTTG TGGATGCTGC AGCTTCTGCA
CCCCAGATGG ATGTGTCCAA AACAAAAGAG AGTAAGCATG AAGCTGCCAG GTTAGAAGAA
AATTTGAGAA GCAGGTCAGC AGCAGTTAGT GAACAGCAGC AGSTAGAGCA GAARAMCCTG
GAGGTGGAGA AGAGATCTGT TCAKTGTCTA TACACTTCTT CAGCCTTTCC AAGTGGCAAG
SCCCAGCCTC ATTCTTCAGT AGTTTATTCT GAGGCTGGGA AAGATAAAGG GCCTCCTCCA
AAATCCAGAT ATGAGGAAGA GCTAAGGACC AGASGGAAGA CTACCATTAC TGCAGCTAAC
TTCATAGACG TGATCATCAC CCGGCAAATT GCCTCGGACA AGGATGCGAG GGAACGTGGC
TCTCAAAGTT CAGACTCTTC TAGTAGCTTA TCTTCTCACA GGTATGAAAC ACCTATCGAT
GCTATTGAGG TGATAAGTCC TGCCAGCTCA CCTGCGCCAC CCCAGGAGAA ACTGCAGACC
TATCACCCAG ATGTTGTTAA AAGGCAAATC AAGCGGAAAA TGATCCTACC AGACAATATG
AAGGACCATT ACATCACTAT CGACCACAGC AGGAATCACC ATCCCCAACA ACAGCTGCCC
CCTTCTTCAC AGGCAGAGGG AATGGGGCAA GTGCCAGGAC CCATCGGCTG ATCACACTTG
CTGATCACAT CTGTCAAATT ATCACACAAG ATTTGCTAGA AATCAAGTTT TCCGTAGATC
AGCTCCCCAG T nucleotide 6380

FIG. 1Q

nucleotide 6602--
CCCATCTCCC CACCCCAGGT TCCGGGTGTG CATGAGAAAC AGGACAGCTT GCTGCTCTTG
TCTCAGAGGG GCGCAGAGCC TGCTGAGCAG AGGAATGATG CCCGCTCACC AGGCAGTATA
AGCTACYYGC CTTCATTCTT CACCAAGCTT GAAAGTACAT CACCCATGGT TAAATCTAAG
AAACCTGAAA TTTTTCCCAA GTGGAACTCC TCTGTTGGAG GTGACTCTGA TATGGCATCT
GCTCACCCAG GAACTGAGAT CTTCAATCTG CCACCAGTTA CTNNNTCTGG CGCAGTCACC
TCTNNANGCC ATTCTTTTGC TGATCCCGCC AGTAACCTTG GGCTGGA nucleotide 6949

FIG. 1R

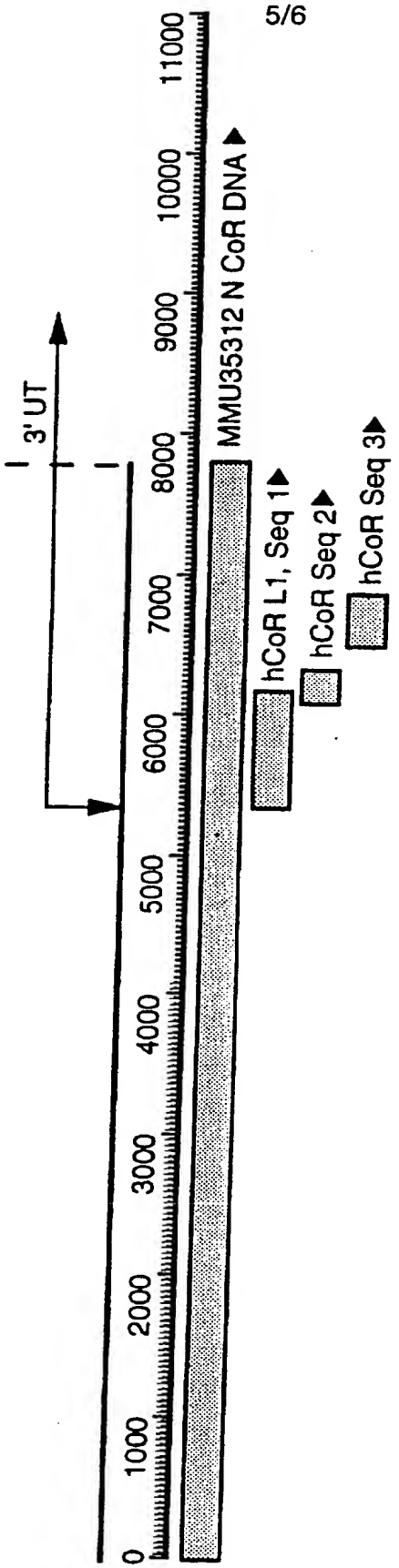

FIG. 2

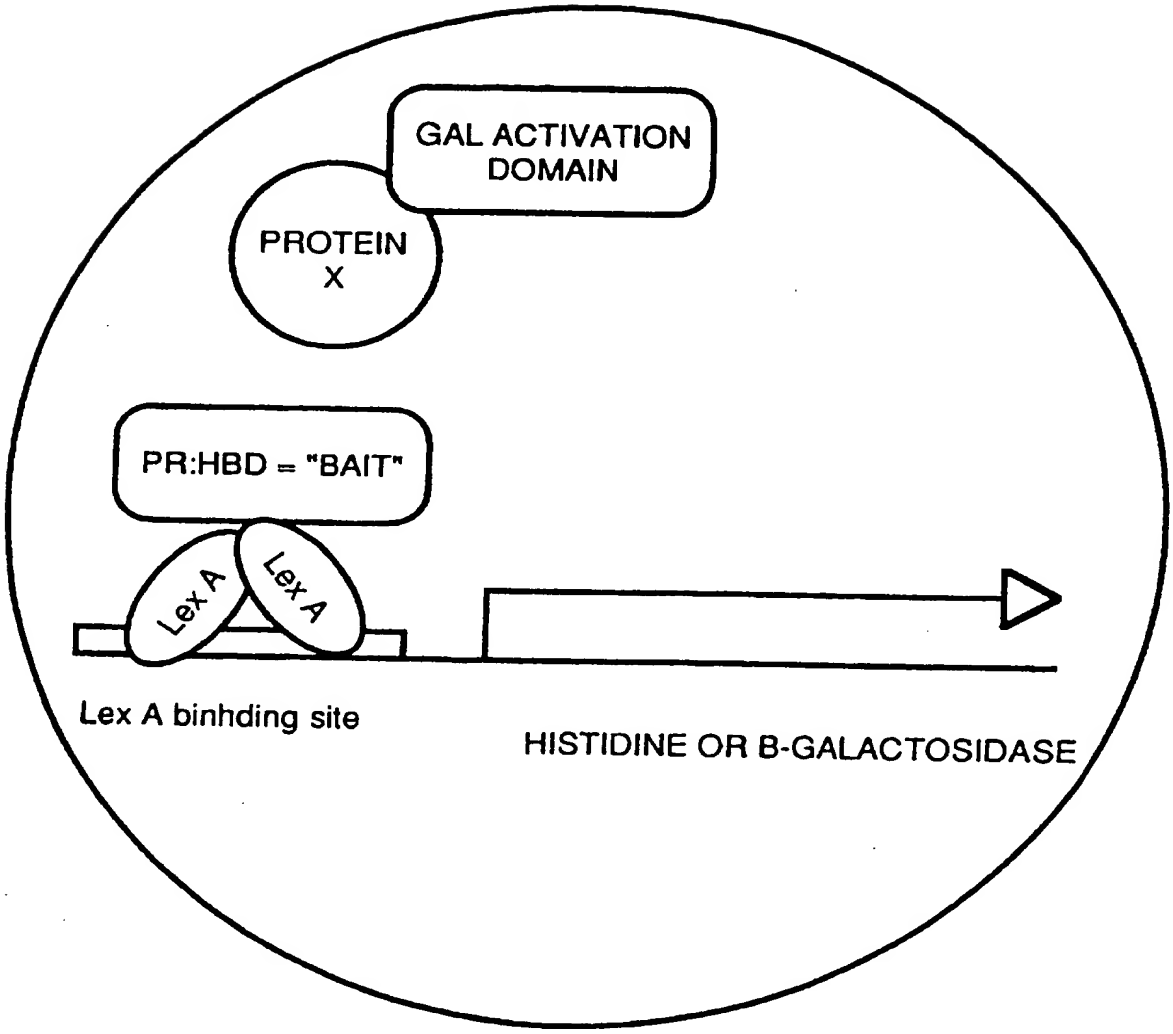

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02954

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/16; C07H 21/04; CO7K14/435; C12N 15/63; G01N 33/566
US CL :435/7.1, 320.1; 514/2; 530/350; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.2, 7.9, 69.1, 91.1, 172.1, 252.3, 254.11, 254.21, 320.1, 325 ; 514/2; 530/300, 350; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	HORWITZ et al. Nuclear Receptor Coactivators and Corepressors. Mol. Endo. October 1996, Vol. 10, No. 5, pages 1167-1177.	1-5 and 9
A	CHEN et al. A Transcriptional Co-Repressor that Interacts with Nuclear Hormone Receptors. Nature. 05 October 1995, Vol. 377, pages 454-457.	1-5 and 9
X	HEMMERICH et al. Strucutral and Functional Properties of Ribosomal Protein L7 from Humans and Rodents. Nucleic Acids Res. October 1993, Vol. 21, No. 2, pages 223-231. Note sequence humL7-14.	1-5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 MAY 1997	Date of mailing of the international search report 09 JUN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THOMAS G. LARSON, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02954

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEOL et al. Isolation of Proteins that Interact Specifically with the Retinoid X Receptor: Two Novel Orphan Receptors. Mol. Endo. January 1995, Vol. 9, No. 1, pages 72-85. Note RP13 sequence.	1-5
Y	HORLEIN et al. Ligand-Independent Repression by the Thyroid Hormone Receptor Mediated by a Nuclear Receptor Co-Repressor. Nature. 05 October 1995, Vol. 377, pages 397-403. Note N-CoR sequence.	1-5

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02954

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7, and 9

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02954

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN (BIOSIS, MEDLINE, CJACS, CAPLUS, WPIDS), Genbank, EMBL, Genseq.

search terms: steroid, receptor, antagonist, modulat?, co-modulat?, cDNA, clone, sequence, Horwitz, K. B., Jackson, T. A. and SEQ. ID. NOS: 1-18.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-5, 7, and 9, drawn to DNA encoding an hNRA-coM, the hNRA-coM protein encoded by said DNA, compositions and cells comprising said DNA, composition comprising said protein, and a method of using said hNRA-coM protein in an assay.

Group II, claim(s) 6, drawn to an antibody to the hNRA-coM protein.

Group III, claim(s) 8, drawn to a method of inhibiting steroid antagonist activity comprising inhibiting hNRA-coM expression.

Group IV, claim 10, drawn to a method of enhancing steroid antagonist activity comprising increasing intracellular hNRA-coM protein levels.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

With regard to group I, the DNA sequence encoding the hNRA protein and the hNRA protein are linked by a special technical feature because the sequence of the protein is determined by the sequence of the DNA. The method of using the protein in an assay is linked to both the protein and the DNA sequence because the assay uses the protein.

With regard to the antibody of group II, it lacks a special technical feature that links it to the compounds of group I because it share a common structural or functional relationship with either the DNA or protein of group I. The fact that the antibody binds the hNRA-coM protein does not constitute a corresponding special technical feature because the antibody would bind (cross react with) any other protein comprising the same epitope. Moreover, many different antibodies may be generated to the same protein.

With regard to the method of Group III, it lacks a special technical feature linking it to group I because the method of inhibiting steroid antagonist activity comprising inhibiting hNRA-coM expression of group III can be practiced using a compound other than that of group I.

With regard to the method of group IV, it lacks a special technical feature linking it to group I because the method of enhancing steroid antagonist activity comprising increasing intracellular hNRA-coM protein levels constitutes a second method of using the protein of group I wherein a first method of using the protein of group I has already been included in group I. The method for determining unity of invention under PCT Rule 13 only provides for, in addition to first product,a single method of using that first product (37 CFR.1.475(d)).